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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/583,277	06/16/2006	Yoshiko Minakuchi	0020-5493PUS1	2615

2292 7590 03/04/2010

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EXAMINER	
NGUYEN, QUANG	

ART UNIT	PAPER NUMBER
1633	

NOTIFICATION DATE	DELIVERY MODE
03/04/2010	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

mailroom@bskb.com

Office Action Summary	Application No.	Applicant(s)	
	10/583,277	MINAKUCHI ET AL.	
	Examiner	Art Unit	
	QUANG NGUYEN, Ph.D.	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *16 December 2009*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-3,5-11,14 and 16-20* is/are pending in the application.

4a) Of the above claim(s) *14 and 16-20* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-3 and 5-11* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicant's amendment filed on 12/16/09 has been entered.

Amended claims 1-3, 5-11, 14, 16-20 are pending in the present application.

Applicants elected previously **siRNA as a species of a nucleic acid**.

Claims 14 and 16-20 were withdrawn previously from further consideration because they are directed to a non-elected invention.

Therefore, amended claims 1-3 and 5-11 are examined on the merits herein with the above elected species.

Response to Amendment

The rejection under 35 U.S.C. 112, first paragraph, was withdrawn in light of Applicant's amendment, particularly with the new limitation "wherein the final concentration of calcium chloride in the medium of step (b) is within the range of 7.1 mM-30.1 mM".

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of

the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Amended claims 1-3, 5-6 and 8-11 are rejected under 35 U.S.C. 103(a) as being unpatentable over Li et al (US 2004/0147475) in view of Haberland et al. (BBA 1445:21-30, 1999), Haberland et al. (Pharmaceutical Res. 17:229-235, 2000) and Lam et al (Biochim. Biophys. Acta 1463:279-290, 2000). ***This is a new ground of rejection necessitated by Applicant's amendment particularly with the new limitation "calcium chloride in the medium of step (b) is within the range of 7.1 mM-30.1 mM".***

With respect to the elected species, Li et al disclose a method for introducing dsRNAs or siRNAs into cells, cell culture, organs and tissues, and whole organisms to attenuate gene expression (see at least the abstract; Summary of the invention, particularly paragraphs 35-54 and claims). Li et al teach specifically that the dsRNA nucleotide sequence is preferably at least about 25 bases and that it can be introudced into a cell in various ways, including liposome-mediated delivery, viral infection, transformation, transfection mediated by calcium phosphate, electroporation among others (paragraphs 39 and 44). In an exemplification Li et al disclose that rat cells were transfected by **overlaying onto the cells with lipid-DNA complexes containing**

dsGFP RNA in serum-free DMEM and incubated for 5 hours at 37 ^{0}C, following by the addition of DMEM (1 mL) with 20% FBS without removing the transfection mixture (see example III on page 12).

Li et al do not teach a cell culture method in which following the step of contacting a nucleic acid with a cell in a medium, further adding into the medium with a volume of a high-concentration solution of a calcium chloride to bring the final concentration of calcium chloride within the range of 7.1 mM-30.1 mM.

However, at the effective filing date of the present application, Haberland et al (BBA 1445:21-30, 1999) already disclosed at least that **the addition of soluble calcium ions (2 mM) or calcium phosphate precipitates to cells after a transfection period enhances transfection of polycation-mediated non-viral DNA transfer systems, and that calcium ion is not needed for non-viral DNA complex uptake** (see at least the abstract; and particularly Figure 3). They specifically stated "Fig. 3 shows the transgene expression following the different procedures of ECV 304 cells. As a control, the cells were transfected as usual with Ca^{2+} present during transfection for 4 h. The transfection medium was then removed and culture medium without Ca^{2+} added for a further 24 h post incubation (a). **Transfection of the cells in the absence of Ca2+** followed by thoroughly washing the cells after the 4 h transfection period to remove the H1-DNA complexes and addition of new culture medium containing 2 mM Ca^{2+} and incubation overnight (c) resulted in transgene expression similar to that observed in (a). At this Ca^{2+} concentration and incubation overnight the cells remained morphologically healthy" (col. 2 at page 25; and particularly Fig. 3).

Moreover, Haberland et al. (Pharmaceutical Res. 17:229-235, 2000) also demonstrated **the importance of the presence of calcium ions in the post-incubation medium after transfection for overcoming serum inhibition in a polycationic or cationic liposomal gene transfer system** (see at least the abstract and Figures 3-4 and 7). They also stated "Fig. 4 shows the transfection efficiencies of neutral H1-DNA complexes **without Ca^{2+} in the transfection medium** at varying serum concentration under different conditions. When increased serum concentrations were present in the transfection medium and 2 mM Ca^{2+}/0.1 mM chloroquine with 10% serum in the postincubation medium, high transfection efficiencies in the total range of serum concentrations were observed" (col. 2 at page 231; see section entitled " Ca^{2+} is not needed for the complex uptake" and Figure 4).

Furthermore, Lam et al also demonstrated that calcium increases the in vitro transfection potency of plasmid DNA-cationic liposome complexes from 3- to 20-fold in a number of different cell lines; and tested the effect with increasing concentration of Ca^{2+} **(0-100 mM)** using an appropriate volume of the 1 M $CaCl_2$ stock solution (see at least the abstract; sections entitled "In vitro transfection in the presence of Ca^{2+}" and "The transfection potency of complexes is increased in the presence of Ca^{2+}"). Lam et al further stated "**[u]p to 20-fold increases in transgene expression were detected at Ca^{2+} concentrations between 5 and 25 mM. Transfection potencies decreased for Ca^{2+} concentrations at 50 mM or greater, where more than 20% reductions in total cellular protein levels were observed, indicating toxicity** (data not shown)" (col. 1 at page 282, last paragraph).

Accordingly, it would have been obvious and within the scope of skill for an ordinary artisan to modify the teachings of Li et al at least with respect to a method for introducing dsRNAs or siRNAs into cells in a cell culture to attenuate gene expression by adding an appropriate volume of a high-concentration calcium chloride solution (concentration of 0.1M or greater; see definition of the term "high-concentration" on page 13, lines 16-20 of the instant specification) to the medium to attain at least a final concentration of calcium chloride in the range of 2 mM - 25 mM following the step of contacting a nucleic acid with a cell, in light of the teachings of Haberland et al (BBA 1445:21-30, 1999), Haberland et al. (Pharmaceutical Res. 17:229-235, 2000) and Lam et al as presented above. With respect to the limitation of dependent claims 10-11, it would also have been obvious for an ordinary skilled artisan to determine and use an aliquot within the range of 1 uL-20 uL of a high-concentration calcium chloride solution (e.g., 1 M $CaCl_2$ stock solution) per 500 uL of the medium to attain the desired final calcium chloride concentration.

An ordinary skilled artisan would have been motivated to carry out the above modifications because both Haberland et al references demonstrated that at least the addition of soluble calcium ions (2 mM) to cells **after a transfection period enhances transfection of polycation-mediated non-viral DNA transfer systems; calcium ion is not needed for non-viral DNA complex uptake; and the presence of calcium ions in the post-incubation medium after transfection can overcome serum inhibition.** Furthermore, Ca^{2+} concentrations between 5 and 25 mM have been used

successfully to enhance in vitro transfection potency of plasmid DNA- cationic liposome complexes without toxicity to cells as taught by Lam et al.

An ordinary skilled artisan would have a reasonable expectation of success to carry out the above modification in light of the teachings of Li et al., Haberland et al. (BBA 1445:21-30, 1999), Haberland et al. (Pharmaceutical Res. 17:229-235, 2000), and Lam et al; coupled with a high level of skills of an ordinary skilled artisan in the relevant art.

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Response to Arguments

Applicants' arguments related to the above rejection in the Amendment filed on 12/16/09 (pages 6-7) have been fully considered but they are respectfully not found persuasive for the reasons discussed below.

Applicants argue basically that in each of the cited references, the calcium salt is added prior to or at the same time as contacting the cells with the nucleic acid and none of the references disclose the feature of the invention that the calcium salt is added after contacting the cell with the nucleic acid. Applicants also argue that the specification shows that nucleic acids could not be introduced into cells when the cells were previously contacted with calcium chloride as done in the cited prior art (see example 2 and Figure 2 of the present application). Applicants further argue that an ordinary skill in the art would not expect that the addition of the calcium solution after contacting the

cells with the nucleic acid would result in a large increase in efficiency of transfer of the nucleic acid into the cells, and that such result is objective evidence of un-obviousness sufficient to overcome any case of prima facie obviousness deemed to be established by these references.

Firstly, please note that the above rejection was made under 35 U.S.C. 103(a) and therefore none of the cited references has to teach every limitation of the instant claims. It is also improper to consider each of the cited references in total isolation one from the others.

Secondly, Haberland et al (BBA 1445:21-30, 1999) stated "Fig. 3 shows the transgene expression following the different procedures of ECV 304 cells. As a control, the cells were transfected as usual with Ca^{2+} present during transfection for 4 h. The transfection medium was then removed and culture medium without Ca^{2+} added for a further 24 h post incubation (a). **Transfection of the cells in the absence of Ca2+** followed by thoroughly washing the cells after the 4 h transfection period to remove the H1-DNA complexes and addition of new culture medium containing 2 mM Ca^{2+} and incubation overnight (c) resulted in transgene expression similar to that observed in (a). At this Ca^{2+} concentration and incubation overnight the cells remained morphologically healthy". Additionally, Haberland et al. (Pharmaceutical Res. 17:229-235, 2000) stated "Fig. 4 shows the transfection efficiencies of neutral H1-DNA complexes **without Ca^{2+} in the transfection medium** at varying serum concentration under different conditions. When increased serum concentrations were present in the transfection medium and 2 mM Ca^{2+}/0.1 mM chloroquine with 10% serum in the postincubation medium, high

transfection efficiencies in the total range of serum concentrations were observed" (col. 2 at page 231; see section entitled " Ca^{2+} is not needed for the complex uptake" and Figure 4). It is abundantly clear that both of the Haberland references teach procedures in which cells were not previously exposed to Ca2+ during the transfection phrase, and that they were only exposed to culture medium containing 2 mM Ca^{2+} in the post-transfection phrase. Furthermore, **it is noted that as written the claims encompass that the cell at step (a) can be exposed or previously exposed to a calcium chloride concentration**.

Thirdly, the examiner has considered the example 2 and figure 2 of the present application. However, the reported results might be specific only to 293 cells under the specific nucleic acid transfer conditions disclosed in example 2. Additionally, please note the breath of the claims as written as already pointed out in the immediate preceding paragraph. Furthermore, both of the Haberland references demonstrated clearly that the cells were successfully transfected regardless whether the cells were previously exposed to Ca2+ in the preincubation and/or transfection (see at least Figure 3 in the Haberland/BBA reference and Figure 1 in the Haberland/Pharmaceutical Res. Reference).

Fourthly, once again at least Figure 3 in the Haberland/BBA reference showed that **the presence of Ca^{2+} in the post incubation phrase resulted in greatly enhanced transfection rates relative to those of cells that were not exposed at all to Ca^{2+} or only exposed to Ca^{2+} in the preincubation phrase** (compare results (c), (b) and (f) in Figure 3).

Accordingly, amended claims 1-3, 5-6 and 8-11 are rejected under 35 U.S.C. 103(a) as being unpatentable over the cited prior art references for the reasons set forth above.

Claim 7 is rejected under 35 U.S.C. 103(a) as being unpatentable over over Li et al (US 2004/0147475) in view of Haberland et al. (BBA 1445:21-30, 1999), Haberland et al. (Pharmaceutical Res. 17:229-235, 2000), and Lam et al (Biochim. Biophys. Acta 1463:279-290, 2000) as applied to claims 1-3, 5-6 and 8-11 above, and further in view of Kubota et al. (US 2004/0052840; IDS). ***This is a new ground of rejection necessitated by Applicant's amendment.***

The combined teachings of Li et al, of Haberland et al. (BBA 1445:21-30, 1999), Haberland et al. (Pharmaceutical Res. 17:229-235, 2000) and Lam et al. were presented above. However, none of the cited references teaches specifically that the nucleic acid is in a form with atelocollagen.

At the effective filing date of the present application, Kubota et al already taught **an efficient preparation for transferring anti-sense oligonucleotides into a target cell that contains a collagen or atelocollagen as an essential component** (see at least the abstract; paragraphs 95, 101-104 and 108-112).

Accordingly, it would have been obvious and within the scope of skill for an ordinary artisan to further modify the combined teachings of Li et al, of Haberland et al. (BBA 1445:21-30, 1999), Haberland et al. (Pharmaceutical Res. 17:229-235, 2000) and Lam et al. set forth above by also utilizing a formulation of a nucleic acid (e.g., dsRNA

or siRNA) containing atelocollagen for attenuating a target gene expression in cells of a cell culture in light of the teachings of Kubota et al.

An ordinary skilled artisan would have been further motivated to carry out the above modification because Kubota et al already taught that the preparation of an anti-sense oligonucleotide containing a collagen or atelocollagen was found to be efficient for transferring anti-sense oligonucleotides into a target cell due to its intrinsic advantages cited in paragraph 112.

An ordinary skilled artisan would have a reasonable expectation of success to carry out the above modification in light of the teachings of Li et al., Haberland et al. (BBA 1445:21-30, 1999), Haberland et al. (Pharmaceutical Res. 17:229-235, 2000), Lam et al. and Kubota et al.; coupled with a high level of skills of an ordinary skilled artisan in the relevant art.

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Response to Arguments

Applicants' arguments related to the above rejection in the Amendment filed on 12/16/09 (page 7) have been fully considered but they are respectfully not found persuasive for the reasons discussed below.

Applicants argue basically that the Kubota reference does not remedy the deficiencies of the other cited references for the reasons already set forth in Applicants' arguments for the rejection of claims 1-3, 5-6 and 8-11 above.

Please refer to the same examiner's responses to Applicants' arguments for the rejection of claims 1-3, 5-6 and 8-11 above. The citation of the Kubota reference is to supplement the combined teachings of Li et al., Haberland et al. (BBA 1445:21-30, 1999), Haberland et al. (Pharmaceutical Res. 17:229-235, 2000), Lam et al for the use of atelocollagen.

Conclusion

No claim is allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Quang Nguyen, Ph.D., whose telephone number is (571) 272-0776.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's SPE, Joseph T. Woitach, Ph.D., may be reached at (571) 272-0739.

To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1633; Central Fax No. (571) 273-8300.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

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/QUANG NGUYEN/

Primary Examiner, Art Unit 1633